

# SOMATIC MUTATIONS IN MYELOYDYSPLASTIC SYNDROME PATIENTS IN THE CONTEXT OF ALLOGENEIC STEM CELL TRANSPLANTATION

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**Disclosure:** The authors have declared no conflicts of interest.

**Received:** 23.02.16 **Accepted:** 13.05.16

**Citation:** EMJ Oncol. 2016;4[1]:122-129.

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## ABSTRACT

Myelodysplastic syndrome (MDS) is a heterogeneous group of myeloid disorders. Allogeneic stem cell transplantation (alloSCT) is the therapeutic approach with a known curative potential for patients with MDS, which allows long-term disease control to be achieved. Despite advances in transplantation technology, there is still a considerable morbidity and mortality associated with this approach. Moreover, numerous controversies still exist regarding alloSCT in MDS. There is significant variability in the management of patients with MDS, especially of the intermediate-risk category and specifically in regards to the timing and use of transplantation. Modern genetic analysis has identified a variety of new mutations, which are associated with clinical phenotype and prognosis. Whether somatic mutations are important prognostic markers of response to alloSCT is little known. It is not clear whether somatic mutations can help to identify groups that are most likely to benefit from alloSCT. In this article, we review the current status of somatic mutations in MDS and focus on the prognostic impact of mutations in the context of alloSCT.

**Keywords:** Myelodysplastic syndrome (MDS), somatic mutations, allogeneic stem cell transplantation (alloSCT).

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## INTRODUCTION

Myelodysplastic syndromes (MDSs) are a clinically and molecularly heterogeneous group of clonal stem cell disorders characterised by ineffective and dysplastic myeloid cell differentiation and a high rate of progression to acute myeloid leukaemia (AML). Treatment options for MDS range from observation and growth-factor therapy to more intensive approaches, such as allogeneic stem cell transplantation (alloSCT).<sup>1,2</sup> AlloSCT is the only therapeutic option that has the potential to produce long-term remission and disease-free survival.<sup>3</sup> Furthermore, introduction of reduced intensity conditioning decreased non-relapse mortality, especially in older patients.<sup>4</sup> Nevertheless, alloSCT is a high-risk procedure that is often associated with severe complications.

The main cause of treatment failure in MDS patients after alloSCT is relapse of disease. Although significantly reduced over the last 20 years, mortality and morbidity risks associated with alloSCT continue to represent a major limit to feasibility in a large number of patients.<sup>5</sup> The international prognostic scoring system and the revised version (IPSS-R) are usually used to determine risk stratification.<sup>6,7</sup> Recently it was demonstrated that alloSCT offers optimal survival benefit when it is performed early in the intermediate-risk stage.<sup>8</sup> Other studies have shown that poor and very poor-risk of cytogenetics within the IPSS-R cytogenetic risk categories are strong predictors of adverse outcomes after alloSCT, especially when other risk factors are considered simultaneously.<sup>7</sup>

**Table 1: Most common somatic mutations in myelodysplastic syndrome patients.**

Mutations	Frequency (%)	Typical clinical phenotype	Prognostic impact	Prognostic impact after alloSCT
<b>RNA splicing machinery</b>				
<i>SF3B1</i>	15-30	RARS	Good overall survival, low-risk of leukaemic evolution	No data
<i>SRSF2</i>	10-20	RCMD, RAEB	Poor overall survival, high-risk of leukaemic evolution	Not shorter OS after alloSCT <sup>37</sup>
<i>U2AF1</i>	5-10	RCMD, RAEB	High-risk of leukaemic evolution	Not shorter OS after alloSCT <sup>37</sup>
<i>ZRSR2</i>	5-10	Subtypes without ring sideroblasts	Shorter OS in co-existing with <i>TET2</i> mutation	No data
<b>DNA methylation</b>				
<i>TET2</i>	19-26	All MDS subtypes	No impact on OS, predict response to hypomethylating agents	Shorter OS after alloSCT <sup>19</sup> No prognostic impact <sup>48</sup>
<i>DNMT3A</i>	5-18	All MDS subtypes	Adverse outcome in RCMD and RAEB, but not in RARS	Shorter OS after alloSCT <sup>19</sup> No prognostic impact <sup>48</sup>
<i>IDH1/IDH2</i>	2-9	RCMD, RAEB	Adverse outcome	No prognostic impact <sup>48</sup>
<b>Chromatin modification</b>				
<i>ASXL1</i>	15-20	RCMD, RAEB	Adverse outcome	No prognostic impact <sup>48</sup>
<i>EZH2</i>	5-8	RCMD, RAEB	Adverse outcome	No data
<b>Transcription factors</b>				
<i>RUNX1</i>	5-15	RCMD, RAEB	Adverse outcome	No prognostic impact <sup>48</sup>
<i>BCOR</i>	2-4	RCMD, RAEB	Adverse outcome	No prognostic impact <sup>48</sup>
<b>DNA repair control</b>				
<i>TP53</i>	5-10	Advanced stage of disease, complex karyotype, often in MDS with deletion of the q arm of chromosome 5	Poor overall survival, high-risk of leukaemic evolution, predicts poor response to lenalidomide in MDS with deletion of the q arm of chromosome 5	Shorter OS after alloSCT <sup>19</sup>

OS: overall survival; MDS: myelodysplastic syndrome; alloSCT: allogeneic stem cell transplantation; RARS: refractory anaemia with ring sideroblasts; RCMD: refractory cytopenias with multilineage dysplasia; RAEB: refractory anaemia with excess blasts.

Further disease characteristics such as peripheral cytopenias and a high percentage of blasts in the bone marrow have been shown to affect the prognosis of patients with MDS after alloSCT.

Moreover, age-related factors significantly affect the risk of the non-relapse mortality.<sup>9,10</sup> Further successful treatment with hypomethylating agents has been shown to be associated with better

outcome after alloSCT.<sup>11</sup> Due to the fact that MDS has a highly variable course of disease, accurate risk stratification and prognosis becomes crucial for therapeutic decision-making. Selection of patients and choosing the optimal timing of transplantation are areas which require further research to improve use of alloSCT in MDS.

The mutation status of a growing number of genes has recently been found to be an important prognostic factor in MDS patients.<sup>2,4,12-14</sup> Although the prognostic value of mutations has been well studied, little is known about the predictive impact of specific genetic lesions in MDS patients on outcome after alloSCT. In this review, we focus on the prognostic impact of somatic mutations in MDS patients in the context of alloSCT.

## MUTATION IN MYELODYSPLASTIC SYNDROMES

Phenotypic heterogeneity of MDS reflects different somatic mutations that cause clonal proliferation and evolution. High-throughput molecular technologies, such as next generation sequencing and high-density single nucleotide polymorphism arrays, provide opportunities for patient stratification and personalised treatment based on individual mutation profiles. Recent genetic analyses have identified a variety of new mutations which present at distinct frequencies in subgroups of patients with MDS.<sup>2,14-17</sup> With the advent of next-generation sequencing recurrent somatic mutations are observed in >90% of patients with MDS, with the number of driver mutations having an independent prognostic impact.<sup>2,4,18,19</sup> Mutations in several genes have been reported to influence overall survival (OS) and risk of disease progression.<sup>2,4,12-14</sup> Knowledge about the nature of genes involved in the mutations may improve the understanding of the evolution and development of MDS.<sup>14</sup> In MDS, somatic mutations affect specific classes of genes. The most common mutations detected in MDS patients include RNA splicing machinery, DNA methylation, chromatin modification, transcription regulation, and DNA damage response (Table 1).

## RNA SPLICING MACHINERY

The editing process during transcription of DNA into RNA, which involves removal of non-coding regions, introns, is called splicing.<sup>20</sup> RNA splicing is the most frequently mutated pathway in MDS.

The most commonly affected spliceosome genes included *SF3B1* (splicing factor 3b subunit 1), *U2AF1* (U2 small nuclear RNA auxiliary factor 1), *U2AF2* (U2 small nuclear RNA auxiliary factor 2), *SRSF2* (serine/arginine-rich splicing factor 2), *ZRSR2* (zinc finger [CCCH type], RNA binding motif, and serine/arginine rich 2), and some others.<sup>16,18,21-24</sup> Splicing factor mutations, particularly in *SF3B1*, *U2AF1*, *SRSF2*, and *ZRSR2*, are present in 50–65% of MDS cases, making them the most common class of mutations in MDS.<sup>18,21,24-26</sup> Mutations in the other spliceosome genes (*U2AF2*, *SF1*, *PRPF40B*, and *SF3A1*) occurred with a frequency of  $\leq 1\%$ .<sup>21,23,25,27,28</sup>

A growing body of evidence demonstrates that splice gene mutations define distinct clinical phenotypes and show preferential associations with mutations targeting transcriptional regulation. Recent findings support the role of spliceosome mutations as early mutations that interact with other clonal and sub-clonal events within the same genes during leukaemogenesis in patients with MDS.<sup>29</sup>

### SF3B1

*SF3B1*, together with other factors, forms the U2 small nuclear ribonucleoproteins complex and binds pre-mRNA. Recurrent mutation of the *SF3B1* gene was found in 20–35% of MDS patients and in 70% of MDS patients whose disease is characterised by the presence of ring sideroblasts (in refractory anaemia with ring sideroblasts [RARS]),<sup>4,18,21,22,30,31</sup> although the clonal dominance of mutations in granulocytic cells suggests that oncogenic effects may not be restricted to the erythroid lineage.<sup>21</sup> It was noted that *SF3B1* mutations were frequently associated with an isolated deletion of the q arm of chromosome 5.<sup>18</sup> *SF3B1* mutations are associated with a better clinical outcome.<sup>4</sup> There are no data about the predictive impact of this mutation on outcome after alloSCT. Given the frequent combination of *SF3B1* mutation with favourable prognostic markers and with good clinical outcome, the presence of *SF3B1* mutations as a single aberration should not be an indication for alloSCT.

### SRSF2

The protein SRSF2 forms part of the spliceosome. This protein is important for splice-site selection, spliceosome assembly, and both constitutive and alternative splicing.<sup>32</sup> *SRSF2* is currently the second most frequently mutated splicing gene in

the myeloid diseases.<sup>18,33</sup> These genetic aberrations are almost always heterozygous missense mutations that specifically occur at the amino acid residue P95. In MDS, an *SRSF2* mutation was detected in approximately 10% of cases. They are most often observed in refractory cytopenia with multilineage dysplasia (RCMD) and refractory anaemia with excess blasts (RAEB), two subtypes of high-risk MDS.<sup>13,27</sup> In chronic myelomonocytic leukaemia (CMML), *SRSF2* is the most commonly mutated spliceosome gene (28–47%) and has been associated with older age, less pronounced anaemia, and a normal karyotype.<sup>25,33</sup>

MDS harbouring an *SRSF2* mutation is associated with a higher rate of transformation to AML and with lower OS compared with MDS with wild-type *SRSF2*.<sup>30</sup> However, more recently, the frequency of complex karyotypes and adverse cytogenetic risk aberrations were found to be higher in patients with *SRSF2* mutations than in MDS patients with other mutations.<sup>29</sup> Although this mutation was found to be associated with a poor prognosis,<sup>30</sup> significant difference in OS between wild-type and mutated *SRSF2* in transplanted patients was not detected.<sup>34</sup>

## U2AF1

*U2AF1* encodes the auxiliary factor for the U2 pre-mRNA splicing complex. Eleven distinct mutations have been reported in the *U2AF1* gene.<sup>28,30,35,36</sup> Most of these mutations occur within the two zinc finger domains of *U2AF1*, with S34 and Q157 residues.<sup>34</sup> Using mouse models it was shown that *U2AF1* mutations drive further mutation.<sup>37</sup> In MDS, *U2AF1* mutations are seen in <10% of patients. Although *U2AF1* mutations have been associated with less favourable OS and a higher risk of transformation to AML,<sup>13,23</sup> it is not clear whether the mutation has independent prognostic impact.<sup>25,29</sup> The prognostic effect is known to depend on certain biological factors as well as a combination of cytogenetics and other mutations.

Both the *U2AF1* and *SRSF2* mutations have been associated with poor outcome.<sup>23</sup> Recently, two groups reported no difference in survival between mutated splicing genes and wild-type Wilms' tumour patients undergoing alloSCT.<sup>19,34</sup> Despite some limitations, such as a heterogeneous cohort of patients, these studies provide preliminary evidence to support the theory that alloSCT may potentially reduce the

effects of certain poor-risk somatic mutations recurring in MDS.<sup>34</sup>

## ZRSR2

*ZRSR2* encodes an essential splicing factor which functions in early spliceosome assembly through direct interactions with *U2AF2*.<sup>38</sup> Mutations of the *ZRSR2* gene are present in 10–15% of MDS cases.<sup>18,28</sup> In contrast to other spliceosome gene mutations, *ZRSR2* mutations occur across the entire length of the transcript. Mutations in *ZRSR2* are more prevalent in MDS subtypes without ring sideroblasts and CMML.<sup>25,28</sup> Some authors have shown that *ZRSR2* mutations are associated with an elevated percentage of bone marrow blasts and higher rates of progression to AML.<sup>25,28</sup> However, this was not confirmed by other studies. Furthermore, it was shown that the prognostic impact of *ZRSR2* mutations depends on the mutation status of *TET2*, the gene most commonly combined with *ZRSR2* mutations.<sup>30</sup> There are no data about the prognostic impact of *ZRSR2* mutations on outcome after alloSCT.

## DNA METHYLATION

Epigenetic regulation refers to methylation of cytosine residues of DNA, biochemical modifications of histones, and the expression of non-coding RNAs.<sup>39</sup> Mutations in the epigenetic modifiers are of particular importance in MDS.

## Ten-Eleven Translocation

The ten-eleven translocation (TET) protein family is responsible for demethylation.<sup>40</sup> In addition, TETs regulate gene expression through modification of chromatin at promoter regions.<sup>41</sup> *TET2* is a tumour suppressor gene, and so loss-of-function mutations support the abnormal haematopoiesis observed in MDS.<sup>42</sup> In MDS, *TET2* mutations affect 19–26% of MDS patients.<sup>43</sup> *TET2* mutations are observed in all types of MDS.<sup>13,44</sup> The clinical implication of *TET2* mutations remains unclear. *TET2* mutations are a neutral or favourable prognostic biomarker<sup>44,45</sup> and have been shown to predict response to hypomethylating therapy in MDS.<sup>43</sup> Some studies demonstrated a higher overall response rate to azacitidine treatment in *TET2* mutated cases with no difference in OS.<sup>46,47</sup>

In MDS patients following alloSCT, *TET2* mutations are associated with shorter OS. Taking into account other risk factors, such as complex karyotype,

high level of bone marrow blasts, donor type, and conditioning regimen, *TET2* mutations together with *TP53* and *DNMT3A*, are significantly associated with shorter OS.<sup>19</sup> However in another study, *TET2* mutations showed no prognostic impact in MDS patients uniformly treated with FLAMSA-busulfan conditioning.<sup>48</sup>

### DNMT3A

DNMT3A (DNA methyltransferases 3A) together with other methyltransferases conducts *de novo* methylation of cytosine residues in CpG islands.<sup>49,50</sup> *DNMT3A* mutations occur in approximately 5–18% of MDS patients with the most common mutation involving R882 amino acid residue.<sup>19,43,51,52</sup> These mutations can be found in all MDS subtypes.<sup>13</sup> *DNMT3A* mutations are associated with older age at diagnosis but not with other clinical features or cytogenetics.<sup>52</sup> In RARS, *DNMT3A* mutations more commonly co-operate with *SF3B1* mutations.<sup>13</sup>

Some studies have shown an adverse clinical outcome with a low survival rate and rapid progression to AML in MDS patients with *DNMT3A* mutations.<sup>52</sup> Moreover, it was found that *DNMT3A* mutations have been associated with poor outcomes in some types of MDS, particularly in RCMD and RAEB.<sup>13,52</sup> In RARS, *DNMT3A* mutations often co-occur with *SF3B1* mutations and have no association with adverse outcome.<sup>13,53</sup>

Bejar et al.<sup>19</sup> have identified this mutation as a risk factor, which significantly reduces the OS after alloSCT. The authors observed an increased risk of relapse and death after transplantation, particularly when other predictive variables were considered.<sup>19</sup> In contrast, Christopheit et al.<sup>48</sup> showed no prognostic impact of *DNMT3A* mutations in patients after alloSCT.

### IDH1 and IDH2

*IDH1* and *IDH2* genes encode cytoplasmic/peroxisomal isocitrate dehydrogenase 1 and mitochondrial isocitrate dehydrogenase 2, respectively. These are NADP<sup>+</sup>-dependent enzymes that catalyse the oxidative decarboxylation of isocitrate to  $\alpha$ -ketoglutarate, generating NADPH from NADP<sup>+</sup>. Both genes function in a crossroads of cellular metabolism, cellular defence against oxidative stress, oxidative respiration, and oxygen-sensing signal transduction.<sup>54</sup> In MDS, somatic mutations in *IDH1/2* occur in 2–9% of patients.<sup>45,55–58</sup> Mutations preferentially affected the *IDH2* gene. Since these mutations were discovered

in early MDS, prior to AML transformation, it has been suggested that *IDH* mutations, especially *IDH2*, may contribute to the pathogenesis of MDS.<sup>56</sup>

The simultaneous presence of *IDH* mutations with other genetic changes is commonly observed in myeloid neoplasms.<sup>57</sup> The close association of *IDH2* mutations with *ASXL1*, *DNMT3A*, and *SRSF2* suggests that *IDH2* mutations can interact with these aberrations in the development of MDS.<sup>57</sup> The prognostic implication of *IDH* mutations in MDS remains unclear. Some studies have shown an adverse clinical outcome in MDS patients with *IDH* mutations.<sup>45,58</sup> Furthermore, two studies demonstrated *IDH1* mutation as an independent predictor of inferior survival.<sup>55,59</sup> Very little is known about the prognostic impact of *IDH1/2* mutations in MDS patients after alloSCT. Only one study has examined this relationship and suggests that there is no prognostic significance of these mutations.<sup>48</sup>

## CHROMATIN MODIFICATION

Two genes involved in chromatin modification and regulation are recurrently mutated in MDS: *ASXL1* (additional sex combs like 1) and *EZH2* (enhancer of zeste 2 polycomb repressive complex 2), which encode chromatin-modifying proteins and interact with the polycomb-group repressive complex 1 and 2. Genomic loss of *EZH2* contributes to overexpression of the Hox gene clusters in MDS through epigenetic modifications.<sup>60</sup> Both mutations are most often observed in higher-risk subtypes of MDS such as RCMD and RAEB.<sup>13,61</sup>

### ASXL1 and EZH2

*ASXL1* and *EZH2* mutations are closely associated with myeloproliferative disorders. In MDS, *ASXL1* mutations are found in 15–20% of patients.<sup>45,61</sup> These mutations are generally associated with signs of aggressive progression and poor clinical outcome.<sup>45</sup> Mutations of *EZH2* occur in 5–8% of MDS patients.<sup>13,45</sup> Previously, it was reported that *EZH2* mutations are a prognostic biomarker, associated with shorter OS.<sup>45</sup> Although there are no reports on the effect of *ASXL1* and *EZH2* mutations on outcome after alloSCT, the overall relationship of these mutations with poor outcome may be important for risk stratifications.

## TRANSCRIPTION REGULATION

Runt-related transcription factor 1 (RUNX1) protein takes part in regulating the expression of multiple

genes involved in normal haematopoiesis.<sup>62,63</sup> *RUNX1* mutations occur in 5–15% of MDS patients,<sup>18,64</sup> and are most often observed in RCMD and RAEB.<sup>13</sup> *RUNX1* mutations are detected during disease progression. Furthermore, this mutation is significantly increased even in secondary AML following MDS. The acquisition of additional mutations in genes such as *MLL-PTD* or *FLT3-ITD* with *RUNX1* appears to be an event precipitating leukaemic transformation.<sup>65</sup> *RUNX1* mutations are associated with unfavourable clinical outcomes.<sup>45</sup> Little is known about the prognostic impact of this mutation after alloSCT. In one cohort of MDS patients uniformly treated with FLAMSA-busulfan conditioning, *RUNX1* mutations showed no prognostic impact.<sup>48</sup>

*BCOR* (*BCL6* corepressor) and *BCORL1* (*BCL6* corepressor-like 1) code for related transcriptional corepressors, that are found tethered to promoter regions by DNA-binding proteins. The proteins interact with several different Class II histone deacetylases to repress transcription. Recently, sequencing of *BCOR* genes in MDS patients identified 2–4% of mutations.<sup>66</sup> *BCOR* mutations are often associated with *RUNX1* and *DNMT3A* mutations. These data suggest that *BCOR* mutations define the clinical course rather than disease initiation.<sup>66,67</sup> In MDS, *BCOR* mutations are associated with adverse outcome, shorter OS, and higher incidence of AML transformation.<sup>66</sup>

## DNA REPAIR CONTROL

Tumour protein p53 (*TP53*) is a tumour suppressor protein that regulates expression of genes involved in cell cycle arrest, apoptosis, senescence, DNA repair, and changes in metabolism.<sup>68</sup> *TP53* is the most frequently mutated gene in many types of cancer. In MDS, *TP53* mutations occur in 5–10% of cases.<sup>13,45</sup> These mutations are most often observed in patients with advanced disease, complex karyotype, or adverse karyotype aberrations.<sup>13,45</sup> MDS patients carrying *TP53* mutation have an unfavourable clinical outcome and a high risk of leukaemic evolution.<sup>4,45</sup>

Bejar et al.<sup>19</sup> have analysed somatic mutations as a potential risk factor in MDS patients following alloSCT. In this analysis, only mutations in *TP53* were significantly associated with shorter OS and progression-free survival. *TP53* mutations are closely associated with complex karyotype. However, in patients with complex cytogenetics,

*TP53* mutation showed significantly shorter OS. In contrast, patients with complex cytogenetics without *TP53* mutation had OS comparable to that of patients without complex cytogenetics.<sup>19</sup> Moreover, there are data demonstrating that *TP53* mutations in low-risk MDS with deletion of the long arm of chromosome 5 treated with lenalidomide are associated with an increased risk of leukaemic evolution.<sup>69</sup> In view of the above, this group of low-risk MDS patients could be candidates for alloSCT.

## CONCLUSIONS

Currently, little is known about the prognostic value of mutations in MDS patients in the context of alloSCT. Several groups studied the role of alloSCT to overcome the negative impact of mutations in MDS patients. Bejar et al.<sup>19</sup> have found that *TP53*, *TET2*, and *DNMT3A* mutations are significantly associated with shorter OS following alloSCT. Importantly, the authors observed no adverse prognostic signs such as cytogenetics or bone marrow blasts percentage before transplantation in *DNMT3A* and *TET2* mutated cases. Nevertheless, after alloSCT these patients had an increased risk of relapse and death, particularly when other predictive variables were considered. In this study, *TP53* mutation status was the most significant predictor of mortality after transplantation.

Christopeit et al.<sup>48</sup> analysed MDS patients treated with FLAMSA-busulfan sequential conditioning and alloSCT. *RUNX1*, *GATA2*, *TET2*, and *CEBPA* and *TP53* mutations were the most frequently observed in this cohort. None of the mutations showed a prognostic impact in this analysis. Recently, Hamilton et al.<sup>37</sup> examined the effect of alloSCT on the negative prognostic impact of *U2AF1* and *SRSF2* mutations in MDS patients. They reported no difference in survival between mutated splicing genes and wild-type patients undergoing transplantation. Thus, the authors suggest that patients with such mutations could benefit from alloSCT.

All reports have some limitations, such as heterogeneity of patients, conditioning, and others. Despite these limitations, some analysis has provided preliminary evidence to support the hypothesis that alloSCT may mitigate the effects of certain poor risk somatic mutations. In general, somatic mutations such as *TP53*, *TET2*, and

*DNMT3A* in MDS patients might be important predictors for adverse outcome after alloSCT.<sup>19,37</sup>

MDS is characterised by mutations in >40 genes. The complex structure of gene crosstalk, multiple mutations, and extensive sub-clonal architecture are common for MDS.<sup>4,25</sup> Recent genetic analysis of large populations revealed some somatic mutations in healthy people in advanced age. These findings support the hypothesis that mutations in genes such as *DNMT3A*, *ASXL1*, *TET2*, and others are likely to be initiating events for MDS and/or AML.<sup>17,70-72</sup> These mutations can confer an increased risk of subsequent

haematological malignancy diagnosis as well as higher all-cause mortality.<sup>17</sup> The discovery of these mutations in elderly patients with low-risk MDS should be considered in risk stratification very carefully.

Accurate risk stratification, patient selection, and timing of alloSCT are frequently difficult in MDS. The decision on whether or not alloSCT is indicated can be challenging. Integration of somatic mutations into prognostic scoring systems may facilitate risk stratification of individual patients and further refine clinical decision-making in MDS.

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